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EVALUATION OF POSSIBLE GOITROGENIC AND ANTI-THYROIDAL EFFECT OF NITRATE, A POTENTIAL ENVIRONMENTAL POLLUTANT

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Abstract : Nitrate is a wide spread contaminant of ground and surface water. The source of nitrate in the ground water may be from run off or seepage from fertilized soil, municipal or industrial waste water, land fills, septic system, urban drainage or decaying plants. Human and animal systems are affected severely on nitrate exposure. The study was to investigate the effect of dietary nitrate exposure on the thyroid status along with the state of iodine nutrition. Rats were fed diet containing 3% potassium nitrate (KNO_3) for 4 weeks and then thyroid status was evaluated by thyroid gland weight, urinary iodine excretion pattern, thyroid peroxidase (TPO) activity, serum levels of total thyroxine (T_4), triiodothyronine (T_3) and thyroid stimulating hormone (TSH) concentrations. In nitrate treated animals, the weight of thyroid gland was increased significantly ($P<0.001$) while thyroid peroxidase activity ($P<0.01$), serum T_4 ($P<0.01$) and serum T_3 levels ($P<0.001$) were reduced; but serum TSH level was increased ($P<0.001$) along with slightly elevated iodine excretion level ($P<0.001$) in comparison to control animals. The overall results indicated the development of a relative state of functional hypothyroidism with enlarged thyroid after nitrate exposure. This study can explain a part for the persistence of residual goitre in the post-salt iodization phase.

Key words : nitrate goitre thyroxine thyroid peroxidase

INTRODUCTION

Nitrate represents the most oxidized chemical form of nitrogen found in natural systems. It is considered as a potential ground and surface water pollutant (1). Consumption of high level of nitrate causes various health problems (2). The source of

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nitrate in the ground water may be from run off or seepage from fertilized soil, municipal or industrial waste water, land fills, septic system, urban drainage or decaying plants (3). It is found naturally in spinach, lettuce, beets and carrot as the main source of nitrate in diet, but small amounts may be present in fish and dairy products (3, 4). In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are first decomposed to ammonia, which is oxidized to nitrite and nitrate. The plants take up nitrate for their growth. Surplus nitrate readily moves with the ground water and contaminate it. The acute oral LD_{50} of potassium nitrate (KNO_3) in rat is 3750 mg/kg body weight (5). Nitrate has the same ionic size and volume as that of thiocyanate and perchlorate anions that mainly inhibit the accumulation of iodide in the thyroid gland (6). Bloomfield et al carried out first principal investigation about the influence of nitrate on thyroid physiology (7, 8). Nitrate toxicity also causes thyroid dysfunction (9). The effect of nitrate on thyroid function is strong if a nutritional iodine deficiency exists simultaneously (10). However, the effect of nitrate exposure on the morphological and functional aspects of thyroid physiology in the state of adequate iodine nutriture is not sufficient. Therefore, the present study was designed to investigate the effects of consumption of nitrate on thyroid status along with the state of iodine nutrition because iodine is an important determinant in the regulation of normal thyroid function (11).

METHODS

Animals and treatment– Twenty young growing Wistar rats weighing 80 ± 5 g were allocated to one control and one experimental group of ten each. Animals were caged in unheated well ventilated stainless steel cages and maintained on laboratory standardized normal diet and water ad libitum with dietary nitrate supplementation in the experimental group. The ethical clearance for the use of animals has been duly obtained from the Institutional Animal Ethics Committee.

Control group of rats was fed normal laboratory diet. Experimental group of rats were fed normal laboratory diet containing 3% potassium nitrate i.e. 3 g KNO_3/100 g of standard diet (6). Both groups of rats were provided with the above respective diet for a period of 28 days.

Feed consumption and body weight were measured every three days. In the last week of the treatment each group of animals were kept in metabolic cages for 24 hours to collect the urine over xylene for the analysis of urinary iodine level. At the end of the experimental period body weights of the rats were recorded and the animals were sacrificed following ethical procedure. Just before sacrifice, blood samples were collected from the portal vein of each rat under ether anaesthesia and the serum was separated for the assay of T_4, T_3 and TSH and kept at –20°C till analysis. Just after sacrifice, thyroid glands were weighed after removing connective tissues and preserved to assay thyroid peroxidase activity (TPO).

Estimation of iodine in urine– It was measured by dry ashing following the method of Karmarkar et al (1986) (12). In this method iodine content in urine sample was estimated by drying urine at 600°C in presence of potassium carbonate and the

iodine present in the ash was measured by ceric-arsenite system.

Assay of thyroid peroxidase activity- Thyroid peroxidase activity of the thyroid tissues was measured following I_3^- from iodide in presence of H_2O_2 in the assay medium by the method of Alexander (13). The tissue protein level was determined by the method of Lowry et al (14) using bovine serum albumin as standard. The results are expressed as ΔOD/min/mg tissue.

Enzyme linked immunosorbant assay of total circulating thyroxine and triiodothyronine– Circulating thyroxine and triiodothyronine levels were measured using Monobind, Inc. total T_4–T_3 kit [kit no. MBI 32718/083001].

Measurement of TSH by radio immuno assay– The serum TSH level was measured by radio immuno assay using radioisotope ^{125}I.

Statistical analysis– Level of significance of all the parameters was determined following independent, unpaired Students' t-test.

RESULTS AND DISCUSSION

Nitrate is used widely as fertilizer. It enters the body mainly through food and water. It has been reported by some earlier studies that nitrate has some differential effects on thyroid status (9). But the effect of nitrate exposure on morphological and functional aspects of thyroid gland is not sufficiently studied. Therefore in the present study an attempt has been made to evaluate the possible consequences of nitrate exposure on thyroid morphology and function.

The results presented here demonstrate that the body weight was reduced non-significantly in nitrate fed rats in comparison to control (Table I). This observation might be due to inhibitory action of nitrate on secretion of growth hormone as had been reported earlier (9). However, the weight of the thyroid gland was increased significantly in nitrate-fed group in comparison to that of control group. The increase in thyroid weight in nitrate-fed rats was due to the increased level of circulating thyroid stimulating hormone (TSH) as observed in the present study (Table I).

Thyroid peroxidase (TPO) catalyses the biosynthesis of thyroid hormone acting at different levels viz. organification of iodide, iodination of tyrosine residues of

TABLE I: Nitrate induced alterations in urinary iodine and thyroid status in 20 Wistar rats

Groups	*Body weight (Initial) (g)*	*Body weight Final (g)*	*Thyroid weight (mg/100 g boty weight)*	*Urinary iodine (μg/dl)*	*TPO activity (ΔOD/min/ mg protin)*	*Serum T4 (μg/dl)*	*Serum T3 (ng/dl)*	*Serum TSH (ng/dl)*
Control	80.33±5.1	98.80±5.90	8.9±0.4	409.35±0.7	0.81±0.2	4.21±0.28	143.5±0.98	0.35±0.07
Nitrate treated	80.66±4.5	96.00±5.10	12.88±0.32*	421.19±1.65*	0.13±0.03*	2.4±0.48**	85.30±0.88*	2.4±0.3*

Values are mean ± SD of ten observations.

*P<0.001; **P<0.01 when compared to control.

thyroglobulin and coupling reaction for the synthesis of thyroxine and triiodothyronine attached to thyroglobulin (15). A significant decrease in the activity of this enzyme was noted in nitrate-fed rats (Table I). The decrease in TPO activity might be due to decrease accumulation of iodide in the thyroid gland due to the interference by nitrate in the iodide uptake mechanism; nitrate also reduces the iodination of tyrosine residues because it has the same ionic size and volume like iodine/thiocyanate (6, 16). However, the iodine intake of nitrate fed group of rats was quite normal as evidenced by urinary iodine excretory pattern as body's 90% iodine is excreted through urine (17). The slightly elevated urinary iodine excretion in nitrate fed rats may be explained by reduced accumulation of iodide in thyroid gland due to the interference by nitrate. Similar finding is noted in rats fed thiocyanate that increases the urinary excretion of iodine (18).

Serum total circulating levels of T_4 and T_3 were assayed in nitrate-fed rats along with the control group. Both the serum T_4 and T_3 levels were decreased significantly in nitrate-fed rats. Similar observation was reported earlier (9). Inhibited TPO activity and decreased accumulation of iodide in the thyroid gland due to nitrate as reported by earlier workers (19, 20) might be the possible reason for the reduced serum T_4 and T_3 concentrations.

Serum TSH level showed a significant rise in nitrate fed rats in comparison to that of the control group (Table I). The decreased serum levels of T_4 and T_3 had stimulated the hypothalamic – pituitary axis by feed back mechanism to increase the secretion of more TSH from pituitary to compensate the thyroid gland function for increased production of thyroid hormone resulting an enlargement of thyroid gland.

The overall results showed that nitrate exposure had increased the thyroid gland weight, reduced TPO activity resulting a relative biochemical state of hypothyroidism as evidenced by reduced serum T_4, T_3 and elevated TSH profiles. Therefore, nitrate possesses goitrogenic and anti-thyroidal activity even in the state of adequate iodine nutrition and this can partly explain the persistence of residual goitre in the post salt iodization phase.

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